

CLINICAL SCIENCE

T-cell large granular lymphocytic leukemia: treatment experience with fludarabine

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OBJECTIVES: The aim of this retrospective study was to investigate the results of T-cell large granular lymphocytic leukemia treatment with fludarabine by assessing the complete hematologic response, the complete molecular response, progression-free survival, and overall survival.

METHODS: We evaluated the records of six patients with T-cell large granular lymphocytic leukemia who were treated with fludarabine as a first-, second-, or third-line therapy, at a dose of 40 mg/m², for three to five days per month and 6 to 8 cycles.

RESULTS: Of the six patients investigated with T-cell large granular lymphocytic leukemia who were treated with fludarabine, five (83.3%) were female, and their median age was 36.5 years (range 18 to 73). The median lymphocyte level was $3.4 \times 10^9/L$ (0.5 to 8.9). All patients exhibited a monoclonal T-cell receptor gamma gene rearrangement at diagnosis. Two (33.3%) patients received fludarabine as first-line treatment, two (33.3%) for refractory disease, one (16.6%) for relapsed disease after the suspension of methotrexate treatment due to liver toxicity, and one (16.6%) due to dyspnea. A complete hematologic response was achieved in all cases, and a complete molecular response was achieved in five out of six cases (83.3%). During a mean follow-up period of 12 months, both the progression-free survival and overall survival rates were 100%.

CONCLUSION: T-cell large granular lymphocytic leukemia demonstrated a high rate of complete hematologic and molecular response to fludarabine, with excellent compliance and tolerability rates. To confirm our results in this rare disease, we believe that fludarabine should be tested in clinical trials as a first-line treatment for T-cell large granular lymphocytic leukemia.

KEYWORDS: Fludarabine; Treatment; Large Granular Lymphocyte Leukemia.

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INTRODUCTION

T-cell large granular lymphocytic (T-LGL) leukemia is characterized by a monoclonal expansion of CD3-positive T-LGL cells, as described in 1975 (1). This rare and indolent disorder represents 2% to 3% of chronic lymphoid leukemia cases, with a median age at diagnosis of 60 years and an equal male to female ratio (2). The diagnostic criterion is a persistent increase in circulating monoclonal large granular lymphocytes lasting at least six months. The neoplastic cells are CD3, CD8 and TCR $\alpha\beta$ in 80% of cases, but they may be

CD4-CD8-, CD4+CD8- or CD4+CD8+ in rare variants of the disorder. The associated natural killer cell antigens (NKA) CD16, CD56 and CD57 are variably expressed; CD57 is the most commonly expressed cell antigen and CD56 is the least commonly expressed cell antigen. A total or partial lack of other T-cell antigens, such as CD2, CD5 and CD7, can be observed (3-4).

T-LGL leukemia is associated with autoimmune disorders, such as rheumatoid factor with or without rheumatoid arthritis, Felty's syndrome, Coombs-positive hemolytic anemia, idiopathic thrombocytopenic purpura, pure red cell aplasia, the presence of positive anti-nuclear antibodies, the presence of anti-neutrophil cytoplasmic antibodies, hypogammaglobulinemia, and polyclonal hypergammaglobulinemia. Anemia and neutropenia are frequent (5). At diagnosis, 70% of patients require treatment, but a watch-and-wait approach may be appropriate for asymptomatic patients (2). The most common indications for treatment are

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cytopenia, recurrent infection and pure red cell aplasia, progressive splenomegaly and B symptoms (5).

There is currently no gold standard treatment for T-LGL leukemia. Most patients are treated with low doses of methotrexate, cyclophosphamide, and cyclosporine-A. As a rule, the treatment should be continuously monitored and, if necessary, adjusted to maintain the desired response. However, treatment compliance is frequently low, with a consequent relapse of the disease. Moreover, side effects are common and may limit the treatment effectiveness (6-7). We therefore changed our approach to treating T-LGL leukemia in an attempt to improve the hematological and molecular response rates and to reduce the treatment duration. Previous studies have shown promising results with fludarabine (8), 2'-deoxycoformycin and alemtuzumab in T-LGL leukemia, with a response rate of 40% to 60% when used as second-line therapy (9). Based on our long-term, positive experience with fludarabine in other hematological disorders and in view of the high cost and toxicity of alemtuzumab, we changed our treatment approach to T-LGL leukemia by adopting fludarabine as the drug of choice. Herein, we report our experience using fludarabine in the treatment of six patients with T-LGL leukemia.

PATIENTS AND METHODS

Patients and end points

This study was a retrospective analysis of data obtained by reviewing the medical records of six patients with T-LGL leukemia. They were treated with fludarabine by the same Hematology Service of the Clinical Hospital of São Paulo and Cancer Institute (São Paulo, Brazil) from January 2007 to January 2009 and February 2009 to October 2010, respectively.

The primary and secondary end points were complete hematologic (CHR) and complete molecular (CMR) response and progression-free (PFS) and overall survival (OS), respectively.

Diagnostic criteria for T-LGL leukemia

The diagnostic criterion for T-LGL leukemia was a persistent (at least six months) increase in circulating T-LGL cells, as evaluated by cell morphology and phenotype and confirmed by a monoclonal T-cell receptor (TCR) gamma gene rearrangement. A complete blood cell count, peripheral blood (PB) slide examination, and phenotype determination by flow cytometry were carried out using a standard multiparameter methodology to assess the antigens CD2, CD3, CD4, CD5, CD7, CD8, CD56, CD16, CD58, TCR β , and TCR $\gamma\delta$, as previously described (10). The equipment used was a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) and the CellQuestPro software. For analysis, the lymphocyte region was delimitated by an electronic gate in the forward side scatter versus side scatter display. Populations of T-LGL cells were defined by the co-expression of the T-cell CD3 antigen and at least one NKa CD57, CD16 or CD56 antigen. The normal range of T-LGL cell numbers assumed in the analysis was $0.1\text{-}0.3 \times 10^9/\text{L}$ (11).

T-cell monoclonality assessment

The monoclonal rearrangement of the TCR gamma gene was assessed by polymerase chain reaction (PCR) using primer sequences, as described previously by Shadrach B and Warshawsky I (12). Briefly, each 25- μl PCR reaction contained 200 ng of DNA, 1.5 U of Taq, 1.5 nmol/L of

MgCl₂, 0.2 nmol/L of dNTP, and 0.5 $\mu\text{mol}/\text{L}$ of each primer (IDT Technologies, Illinois, USA). PCR was performed in a PTC-100 DNA Engine Tetrad (MJ Research, Inc., Waltham, MA) with one cycle at 94°C for three min, followed by 40 cycles at 95°C for 60 sec, 61.8°C for 30 sec, and 72°C for 30 sec, with a final extension of 10 min at 72°C and then held at 4°C. A 0.1- μl aliquot of the PCR product was mixed with 12 μl of deionized formamide and 0.5 μl of GeneScan 500 HD Rox size standard (Applied Biosystems, Foster City, CA). Then, the mixture was injected into an ABI Prism 3130 Genetic Analyzer, and the resulting data were analyzed using the GeneMapper V 3.2 software (Applied Biosystems, Foster City, CA). A monoclonality spike was determined by visual examination of the electropherograms (12).

Treatment

Patients with T-LGL were treated with fludarabine if they presented with B symptoms, progressive splenomegaly, recurrent infection or anemia. Fludarabine was used as a first-line treatment or salvage therapy. HIV-positive patients were excluded from the study.

Fludarabine was used at a dosage of $40 \text{ mg/m}^2/\text{day}$ pathway oral for three to five days monthly for 6/8 cycles. Granulocyte colony-stimulating factor was given to all patients beginning from the 10th day of each cycle until the end of the cycle, and it was given for five days if the neutrophil count was less than $1.0 \times 10^9/\text{L}$ because it was not possible to differentiate between neutropenia secondary to the disease or due to drug toxicity. Prophylaxis for *Pneumocystis jiroveci* with trimethoprim-sulfamethoxazole was also indicated for all patients.

Assessment of treatment response

The treatment response was evaluated by periodic clinical assessments, complete blood cell counts and phenotype and molecular analysis after cycles four, six and eight. CHR was defined as the complete normalization of blood counts (i.e., hemoglobin $>120 \text{ g/L}$, platelets $>150 \times 10^9/\text{L}$, and neutrophils $>1.5 \times 10^9/\text{L}$) and the absence of T-LGL cells. Partial hematologic response (PHR) was defined as an improvement of more than 50% in the complete blood cell count. CMR was defined as the absence of a monoclonal population as assessed by PCR (13).

RESULTS

Clinical features

Five (83.3%) patients were female, and the median patient age was 36.5 years (18 to 73) (Table 1). The median lymphocyte level was $3.4 \times 10^9/\text{L}$ (0.5 to 8.9), with a median T-LGL cell number of $2.2 \times 10^9/\text{L}$ (0.4-5.3). The median neutrophil count was $0.35 \times 10^9/\text{L}$ (0.2-0.8). Five (83.3%) patients had anemia, with a median hemoglobin level of 105 g/L (38-143); two patients (33.3%) presented with pure red cell aplasia, and the disease was associated with Felt's syndrome in two patients (33.3%) (Table 1). Four (66.6%) patients were CD3+CD8+TCR $\alpha\beta+$, one (16.6%) was CD4+CD8-TCR $\alpha\beta+$, and one (16.6%) was CD4-CD8-TCR $\gamma\delta+$. Five (83.3%) patients were CD57+CD16+, and none of them were CD56+. All patients were positive for the monoclonal TCR gamma gene rearrangement.

Two (33.3%) patients received fludarabine as first-line treatment, another two (33.3%) for refractory disease, one (16.6%) for relapse disease after discontinuing methotrexate treatment due to liver toxicity, and one (16.6%) due to

Table 1 - Clinical Characteristics of Patients.

Patient	Age/gender	Presentation	Hemoglobin (g/L)	Lymphocytes ($\times 10^9/L$)	Neutrophils ($\times 10^9/L$)	Platelets ($\times 10^9/L$)
1	54/F	Felty's syndrome	143	1.8	0.3	147
2	45/M	Infection	117	3.9	0.4	319
3	73/F	Felty's syndrome	129	0.5	0.2	245
4	25/F	Infection	105	8.9	0.8	202
5	28/F	PRCA	38	1.6	0.8	147
6	18/F	PRCA	69	3.4	0.3	172

F = Female; M = Male; PRCA = pure red cell aplasia.

dyspnea. The most common sign of toxicity was grade 4 neutropenia without infection.

CHR was achieved in all cases, and CMR was achieved in five out of the six patients (83.3%) (Figure 1). No case of hematological relapse occurred within a median follow-up time of 12 months. All patients are still alive. Bone marrow transplantation was not performed after the treatment with fludarabine because T-LGL is an indolent disease and is associated with a high rate of mortality and morbidity.

DISCUSSION

Herein, we describe our experience concerning T-LGL leukemia treatment with fludarabine. We observed a high rate of CHR and CMR, with good compliance and minimal side effects. Because T-LGL leukemia is a rare disease, prospective, randomized studies to evaluate different treatment protocols are not feasible. Therefore, the treatment of T-LGL leukemia is based on the findings of case reports and retrospective studies (14-15). Thus, until 2006, cyclosporine and methotrexate were the only first-line therapy options for T-LGL leukemia. Although effective, maintenance therapy is required to achieve sustained responses with these drugs. However, maintenance therapy results in significant side effects, especially in younger patients (7,14,15). In fact, in our institutions, the median age of T-LGL leukemia patients was less than described in the literature. Therefore, a short time of therapy could certainly produce better results because it avoids adverse events. Although indolent diseases, such as T-LGL leukemia, require a long follow-up period for the treatment to lead to a better overall survival, our experience

showed that with cyclosporine-A and methotrexate, only a minority of patients were able to achieve better results, due to low compliance rates and drug toxicity.

Fludarabine has been used to treat malignant lymphoid disorders since the 1990s (16) and has shown high efficacy in indolent diseases, such as chronic lymphocytic leukemia and non-Hodgkin lymphoma. In 1994, Witzig et al. (17) described a case of T-LGL leukemia with transfusion-dependent anemia that was successfully treated with four cycles of fludarabine after a seven-year history of partial response to several drugs. This patient became transfusion-free, with a partial molecular response lasting more than 15 months. Another study showed CHR in four symptomatic patients treated with fludarabine but with no CMR (8). In another report including 26 patients (14), 19 (73%) were treated with different drugs, either alone or in combination, such as cyclosporine-A, erythropoietin, granulocyte colony-stimulating factor, prednisone, alemtuzumab, pentostatin, fludarabine, cyclophosphamide and infliximab, in addition to splenectomy and hematopoietic stem cell transplantation. Cyclosporine-A was the most commonly used drug; six patients achieved CHR, but none of them achieved immunophenotype CR or CMR. Although the authors reported an absence of response in two patients treated with fludarabine, they had been previously treated at another institution and laboratory data were not available to confirm the diagnosis.

In contrast, the best results concerning CMR in T-LGL leukemia were reported in patients treated with fludarabine, mitoxantrone and dexamethasone (18). Based on this study, we decided to use fludarabine to treat our T-LGL leukemia patients. Our results demonstrated a high rate of CHR and

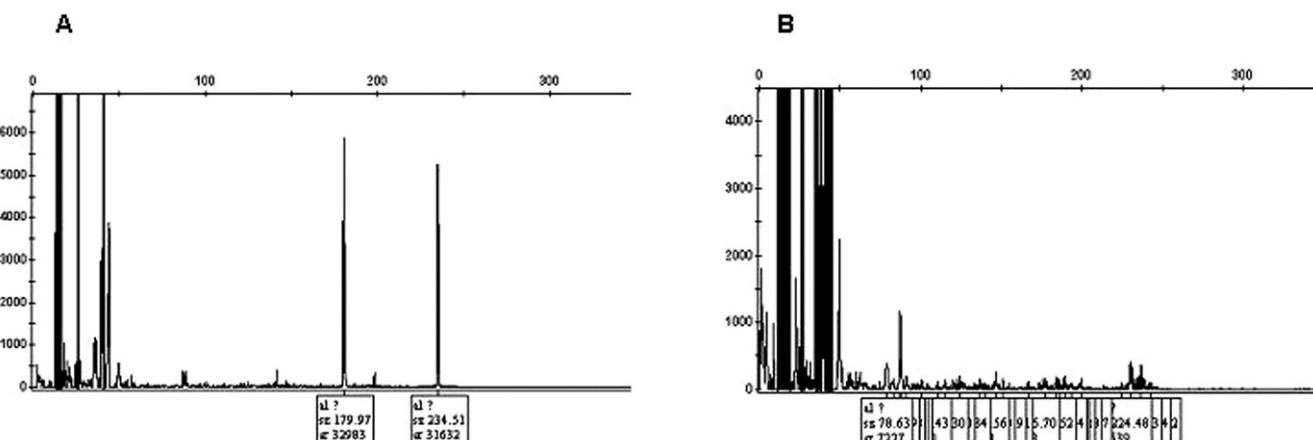


Figure 1 - Peripheral blood of patient 4: (A) at diagnosis, showing T-cell receptor gamma gene monoclonal rearrangement; and (B) after eight cycles of fludarabine, showing that the monoclonal cells were completely replaced by polyclonal T-cells, which is characteristic of a complete molecular response (CMR).

CMR, along with excellent treatment compliance and drug tolerance. In accordance with the literature, our main indication for treatment was cytopenia and recurrent infection (19).

In our series of cases, we found a high rate of CHR and CMR with fludarabine, along with excellent compliance and tolerability. Even though our number of cases was small, we suggest that fludarabine should be further tested as a first-line treatment option for T-LGL to confirm these preliminary data.

AUTHOR CONTRIBUTIONS

Bellesso M and Pereira J were responsible for the data review, manuscript writing and review. Oliveira RC was responsible for the data collection and review, manuscript writing and review. Chamone DAF was responsible for the manuscript review. Ruiz MA was responsible for the manuscript writing. Hallack AE was responsible for the manuscript writing and review. Aldred VL was responsible for the diagnosis review.

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